E. M. Johnson & W. D. Valleau

Kentucky

Agricultural Experiment Station

University of Kentucky

Black-Stem of Alfalfa, Red Clover and Sweet Clover.

BULLETIN NO. 339

(Research Bulletin)

Lexington, Ky.

May 1, 1933.

disease has caused severe damage to each of these crops. A similar and probably identical disease of alfalfa was reported by Rosella (4) as attacking alfalfa in France in 1929. The stands in some cases were considerably reduced by whole plants or the aerial parts being killed. The attacked stems bore numerous pycnidia measuring 160 by 70 microns, on the average. The cause of the disease was attributed to *Ascochyta medicaginis* Fuckel.

In April, 1931, a disease occurred on alfalfa in Italy which produced a necrotic zone at the base of the leaf stalks, causing the leaves to yellow and wither. Blackish, elongated spots were frequently present on the stalks. The floral shoots were occasionally withered. Corneli (1) considered the disease to be distinct from that described by Rosella, but attributed it to the same organism, *Phyllosticta medicaginis* (Fuck.) Sacc. (*A. medicaginis* Fuckel). Horsfall (3, p. 79) reported black-stem to be injurious to red clover in New York. Dr. F. R. Jones sent to the writers cultures of organisms which he had isolated from blackened stems of alfalfa collected in Wisconsin, Nebraska and Mississippi, and from black lesions on sweet clover specimens collected in Wisconsin, Illinois and Ohio.

This disease appears to cause the greatest injury to alfalfa in Kentucky during the early spring, the injury commencing as soon as growth starts and continuing until the first cutting. It is particularly severe following open winters and during long, wet springs. Under these conditions, stands of alfalfa may be greatly reduced or the early crop of shoots killed back, forcing new crown buds to push out from below the soil level.

Principal injury to sweet clover is caused in the spring of the second year. The leaves and young shoots may be so heavily infected that the plant is killed, or the attack may be mild resulting in leaf spotting and a few blackened areas on the stems. Another type of failure of sweet clover appears to be due to the black-stem organism, but the evidence is largely circumstantial. Volunteer stands in sweet clover stubble often die soon after germination. Death of the seedlings appears to be caused by the great accumulation of inoculum on the sweet clover debris which remains on the ground.

Adapted varieties* of red clover are but little affected, the stems blackening to some extent and petiole and leaf spots gradually killing the lower leaves. Unadapted clovers, especially European and some northern varieties, may be much injured by gradual loss of leaves caused by leaf and petiole spots which form during the fall, late winter, and spring. Plots on which these clovers were sown sometimes appear nearly bare in the spring, (Figure 3) the roots and crowns and a few weakened buds often being all that remain. If only adapted varieties of red clover were grown in Kentucky, the disease would be considered a minor one of that crop; at present it is a major disease on commonly grown varieties of clover and alfalfa.

SYMPTOMS

Alfalfa. The most commonly observed symptom is the smooth, black discoloration of the lower portion of the first-crop stems (Figure 1). In heavy stands or in stands not cut at the usual time, blackening may advance well up the stem. The stems of alfalfa which is not cut during the summer become blackened thruout. Blackening of hardened stems does not seem to be very injurious, except that lateral buds and leaves in the blackened area are weakened or killed. Stipules on blackened stems usually wither and turn brown. A more injurious phase of the disease occurs soon after growth commences in the spring. To comprehend the type of injury which occurs at this stage, one should be familiar with the crown of the alfalfa plant. In the fall, the green shoots die to within an inch or two of the crown. By spring, the dead shoots are fruiting heavily with *Phoma.* The bases of these shoots, which usually remain alive over winter, bear from one to several lateral buds capable of growth. The live buds highest on the stems commence growth as soon as conditions permit. Thus the new spring growth arises from the living bases of shoots whose tops are dead. There is no sharp line between dead and living tissue

* Adapted varieties, as used in this Bulletin, are varieties which have been grown locally for many years and are, as a consequence, resistant to various disease-producing fungi and insects which cause failure of unadapted clovers in this vicinity.

and, after the beginning of growth, decay of the old shoots continues, thus progressively destroying the new axillary shoots. Plants starting into growth, if removed to the greenhouse in the

Figure 1. Black-stem of alfalfa. A. Blackened shoots, natural infection, photographed April 24, 1932. B. Alfalfa plants showing numerous blackened and dead shoots. These plants were from a field in which the majority of plants were killed by the disease. Photographed April 27, 1932.

spring, develop this type of injury to a marked degree. Frequently all the new shoots produced from buds above ground, die because of decay of the older shoots upon which they are borne (Figure 2). The only shoots that survive are those produced from below the ground level (Figure 2).

Direct infection of leaves, petioles and shoots begins as soon as growth commences in the spring. This results in death, first, of the leaves and petioles, and gradually of the less vigorous shoots. This type of injury, together with that previously described, may be sufficient to destroy stands in poor soil during a wet spring.

The winter of 1931-1932 was exceptionally mild. Alfalfa which came thru the winter in excellent condition and started growth early, was killed back to some extent by the freeze of

Figure 2. Black-stem of alfalfa. A. The spring growth had all been killed. Buds were alive below ground. Note the overwintered shoots fruiting heavily with Phoma medicaginis. The majority of plants in the field were affected this way. Photographed May 3, 1933. B. Alfalfa plant showing the origin of vigorous shoots from crown buds. Shoots arising from axillary buds have died.

March 9. It again started into growth, but soon the succulent shoots, now outgrown by winter weeds, began to die. Soon in some fields few living plants remained (Figure 1). The extent of injury in one field of alfalfa several years old growing on fertile soil on the Experiment Station farm is indicated by counts made May 5, 1932, by Dr. E. N. Fergus (Table 1.) An examination of the roots of the dead and living plants showed them to be well supplied with starch. Many shoots had started on each plant, but the majority died when but a few inches high. The field was plowed because so few plants were living.

TABLE 1. Injury to alfalfa plants by black-stem during spring of 1932. Counts made in square-yard quadrats.

Quadrat	Number of plants		
	With 6 or more shoots over 4 inches tall	With 4 shoots or less 1 to 4 inches tall	Dead
1	2	44	25
2	2	36	11
3	4	80	36
4	2	40	18

The spring of 1933 was exceptionally wet, a highly favorable condition for the development of black-stem. It was evident thruout the spring that much injury would result to the young shoots of alfalfa, especially those from axillary buds. On April 21, fifteen plants were collected at random from a planting in its third year, on the Station farm at Lexington. At that time alfalfa was well started, averaging about ten inches in height. A count of the dead and dying shoots and the live, vigorous shoots gave the following results:

Number of Plants	Living, vigorous shoots		Dead shoots	
	Total	Ave. per plant	Total	Ave. per plant
15	89	5.9	248	16.5

When the vigorous shoots were gathered together as in Figure 2B it was apparent that nearly all had originated from crown buds and not from axillary buds. The old, dead shoots (Figure 2A) evidently serve as a source of inoculum for *Phoma medicaginis.* It would be an advantage to the spring growth if these shoots were removed by grazing, or by other means during late fall or winter. Blackening of the vigorous shoots at the time the records were made was insignificant, altho numerous minute lesions were present which soon would have caused extensive blackening. Two or three weeks previous to the time these counts were made, blackened shoots were numerous, but all died in the intervening period.

Some alfalfa stands were greatly reduced in Central Kentucky by the first of May, 1933. One of these fields in which the majority of plants were dead or so severely injured that recovery was unlikely, was carefully examined (Figure 2). Most severe injury occurred in the part of the field which produced the heaviest yield in former years. The feature in this field which differed from other fields in which injury was less extensive was the large number of large dead shoots standing among the new growth. As these were fruiting thruout with *Phoma medicaginis,* they furnished a continuous source of inoculum for the new leaves as they developed. For this reason but few shoots could grow above the inoculum and death of the most vigorous shoots finally resulted.

Red Clover. The most conspicuous symptom on red clover is the blackened stems the second year; but this accounts for but a small part of the injury, especially to unadapted clovers. During the first summer, small, black spots develop on petioles and leaves of spring-sown clover, which increase in size slowly, but eventually kill the leaf. This results in the accumulation of a mass of dead leaves by winter. This type of injury appears to be greatest on European, less on northern clovers, and least of all on adapted and southern clovers. During the cold weather of December and January, the remaining foliage dies on all the red clovers. In warm periods in January and February, new foliage puts out which, in wet periods, becomes heavily spotted.

Isolations from these leaf spots have yielded *Phoma* and an unidentified organism which produces a nearly continuous layer of large, black, perithecium-like bodies in culture, but which, so far, have not been observed to produce spores. Leaf and

Figure 3. Effect of black-stem on three varieties of clover. Left, Tennessee 19959; center, French 15787; right, Kentucky 101. The stands December 1, 1932, were 100, 90, and 100 per cent, respectively. One hundred per cent equals seven plants per square foot. Photographed April 23, 1933.

petiole spots continue to develop during wet periods thruout late winter and spring, and gradually kill the older leaves. Again the unadapted clovers are more affected than adapted varieties, leaves on some being killed nearly as rapidly as produced, finally causing death of plants. This type of injury was so severe on certain unadapted clovers in the spring of 1933 that by the first of April plots which had had a perfect stand the previous December were bare (Table 2, Figure 3). A comparative study of the condition of a French and an adapted clover April 14, 1933, is given in Table 3.

Under less favorable conditions for the development of the disease, unadapted clovers in their second year are injured to a less extent and a majority of the plants live. The injury to the first shoots may, however, be so great as to cause their

death; then, what would normally be the second-crop shoots appear. After harvest of the hay crop, these clovers usually die out completely. Adapted clover growing under the same conditions is affected but little, only an occasional shoot being

TABLE 2. The effect of Phoma on stands of adapted and unadapted red clovers during the winter and spring of 1932-33. Records made April 12, 1933. Stand December 1, 1932, 90 percent or over, except where marked,* where stand was 60 percent.[1]

Clover	Vigor	Stand
Tenn. 19959[2]—Ck.	9	90
Kentucky 101	9	95
Virginia 19946	8	85
N. Carolina 16048	8	85
Indiana 18992	7	85
Polish 86782	8	80
Ohio 19950	7	70
Michigan 16120	7	60
*Idaho	4	20
*Oregon 19127	4	5
*Washington 18988	3	2
English 18985	5	15
*Russian 15881	5	20
French 15787	2	2

[1] On April 21, detailed examinations were made of plants which had died out in plots during the spring, to note the extent of injury from Sclerotinia trifoliorum. Very few sclerotia were found, and certain red clover varieties susceptible to Sclerotinia but resistant to Phoma were found to have excellent stands.

[2] The clovers designated by large numbers were furnished the Agronomy Department by Dr. E. A. Hollowell, of the Bureau of Plant Industry, in connection with cooperative studies of red clover varieties.

TABLE 3. Comparative injury by Phoma trifolii to a foreign and an adapted red clover, April 14, 1933. Counts were made on square-yard areas. Stands were nearly equal December 1, 1932.

Variety of Clover	Plants Dead		Plants Alive				
	No Sclerotia	Sclerotia Present	No Foliage	Slight Foliage	Medium Foliage	Excellent Foliage	Total
French 15787	51	4	6	14	9	0	84
Kentucky 101	5	1	1	37	48	94	186

killed and others blackened somewhat. Usually they survive and produce a seed crop, and may have a fair stand the following spring. If clover is allowed to stand after the proper time

Figure 4. A plant of Russian red clover affected by black-stem. The shoots are blackened thruout and most of the leaves have been killed. Photographed August 10, 1932.

for hay harvest, blackening of the stems increases. The stems of the second crop, if allowed to go to seed, become blackened (Figure 4).

The symptoms of plants killed by *Phoma* during the spring of their second season differ from those killed by *Sclerotinia trifoliorum*. In the former the leaves die one by one and decompose, leaving a crown with perhaps a live leaf or two or a small

mass of dead tissue. If this dead tissue is removed, the root will be found intact, altho discolored when split open. It gradually decays. Until completely decayed, the crown does not separate readily from the root. Plants killed by *S. trifoliorum* will be found to have the complete mass of withered shoots and leaves remaining, which separates readily from the root. The presence of sclerotia in the crown makes diagnosis certain.

Sweet Clover. Blackening of the second-year stems, usually the lower few inches, but sometimes covering the entire stunted plant by early May, is the symptom most commonly observed on sweet clover (Figure 5). Buds and leaves in the affected areas are usually killed. Blackening is more abundant in thick, rank stands of sweet clover than in thin stands or on individual isolated plants.

Stone (6) states that *Ascochyta lethalis* (the organism which appears to be the cause of black-stem of sweet clover) attacks plants in their seedling stage and also plants of the second season's growth. He says that "very frequently the stems become girdled and then the upper parts of them die. This takes place most frequently in young seedlings and early in the season of the second year's growth. The more mature plants are seldom killed and the diseased areas may become spread over nearly the whole upper part of the stem including the small branches in the flower clusters." He makes no mention of general blackening of the stems but states that "when infection first takes place, dark areas are formed on the green stems. Then as the fungus spreads the spot becomes oval to elliptical with a grayish white center and a brown raised border. The brown pycnidia appear scattered over the whitish area. The spots may become confluent and very extensive." Girdling and death of seedling plants in early summer, and the whitish spots bearing pycnidia have been observed in the present studies but at first were not thought to be associated with black-stem. Pycnidia have not been observed on the darkened areas while the stems were still alive.

A leaf-spot develops on sweet clover leaves in contact with overwintering stems soon after growth commences in the spring

(Figure 5). The spots are at first dark but as they enlarge turn light brown. When placed in a damp chamber *Ascochyta* pycnidia develop in a few hours. Leaf spotting is much in-

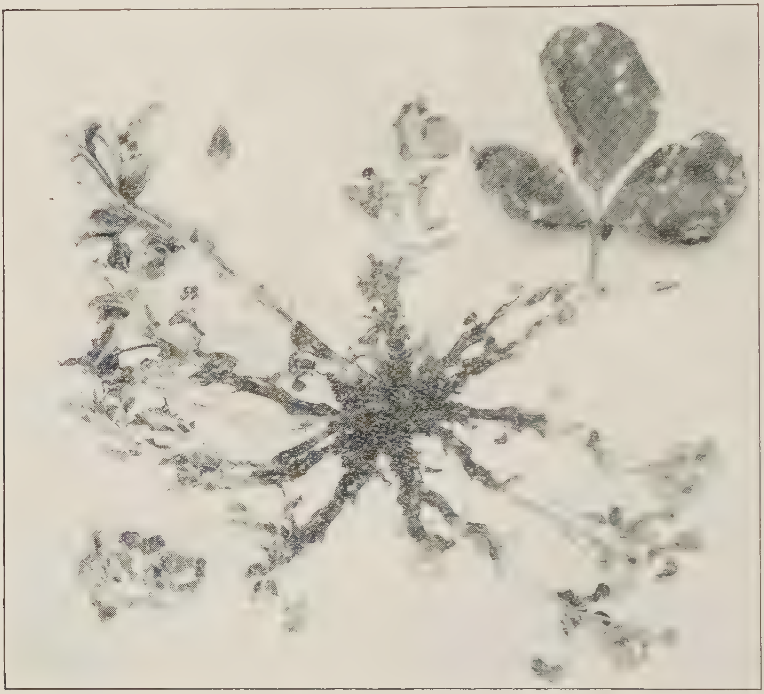

Figure 5. Black-stem of sweet clover. The shoots have been separated to show the extent of blackening. Inset. Sweet clover leaf from the same plant, with leafspot. Ascochyta lethalis was isolated from leafspots and blackened stems.

creased by a type of frost injury which causes the lower epidermis of young leaves to separate from the mesophyl cells. This appears to give an easy means of entrance for the fungus and severe leaf spotting follows.

Heavy stands of sweet clover may be greatly injured by this disease, if the season is wet while the first 6 or 8 inches of growth are being made. The lower leaves may be destroyed and in some instances leaf injury and stem infection are sufficient to kill the shoots, and the plant dies. Just what part this

disease plays in the total destruction of stands of sweet clover the spring of the second year, as sometimes occurs in Kentucky, has not been determined.

THE ORGANISMS

The cause of black-stem differs with each of the three crops, altho in inoculation studies each organism produces blackening of the stems of any of the three species of plants.

Alfalfa. *Phoma* was isolated from leaves, stipules, petioles, blackened stems, and from pycnidia on dead stems of alfalfa in late winter. It may be isolated at any time of the year with the possible exception of a period in the winter after the green parts have been killed by cold and before pycnidia have developed on dead shoots. A simple method of isolation is to place spotted leaves collected during the spring on moist, sterile filter paper in a culture dish. As the leaf dies *Phoma* almost invariably fruits on it and on the paper in the vicinity. Pycnidia may then be picked off and the spores streaked on the surface of agar and single spores picked. It is also readily isolated by plating small bits of affected tissue on acid agar. On potato dextrose agar the organism starts as a white mycelium which soon changes to dark olive-gray. Pycnidia are usually produced in abundance on this medium, and dark-colored heavy-walled chlamydospores are produced, either singly or in chains. Newly isolated cultures of the fungus differ in culture from *Phoma* of red clover and *Ascochyta* of sweet clover in that the latter two organisms almost cover the surface of a slant of potato dextrose agar with a nearly resupinate, white to faint pink, irregularly concentric growth, before the olive-gray mycelium has developt to any extent. Old cultures of the three organisms are much alike except that cultures of the red clover and sweet clover organisms are more likely to be covered with a whitish to gray aerial mycelium with fewer pycnidia while the surfaces of the alfalfa cultures are darker and finally become black and leathery from the abundance of pycnidia and masses of heavy-walled chlamydospores. This organism, in common with the

other two, produces crystals on potato dextrose agar, both in test tubes and in culture plates (Figure 6). The crystals usually appear in about 7 to 10 days. Sometimes they fail to develop, even after several weeks.

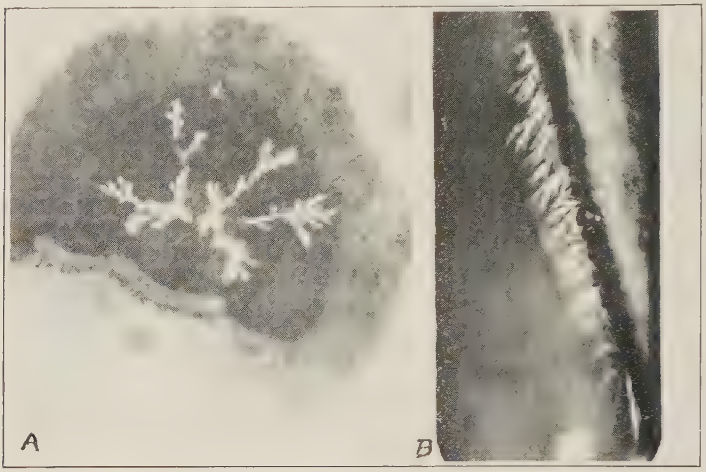

Figure 6. A. A 27-day-old culture of Phoma medicaginis on potato 2 percent dextrose agar, showing crystal production and numerous pycnidia. An interesting example of antagonism between a bacterial contaminant and the fungus is also shown. B. A 21-day-old test tube culture of Phomo trifolii showing crystal production. The same type of crystals is produced in cultures of Ascochyta lethalis.

Pycnidia develop on recently killed stems and stipules of alfalfa in the spring. They have not been found on living blackened stems. On alfalfa leaves in culture dishes they are at first light tan but with age turn dark brown to black. The pycnidia produced on sterile leaves are from 100 to 280 microns in diameter, but average smaller on overwintering stems. When wet on a slide, the spores flow out in a gelatinous, cylindrical or ribbonlike mass, depending on the shape of the ostiole. Spore masses in a damp chamber are light pink and glisten. The spores are hyaline, varying in size from 5 x 1.5 to 12 x 2.5 microns. A few are one-septate. They may be distinguished from the spores of the red clover *Phoma* in that the latter tend to be more uniform in shape and size and are distinctly oval,

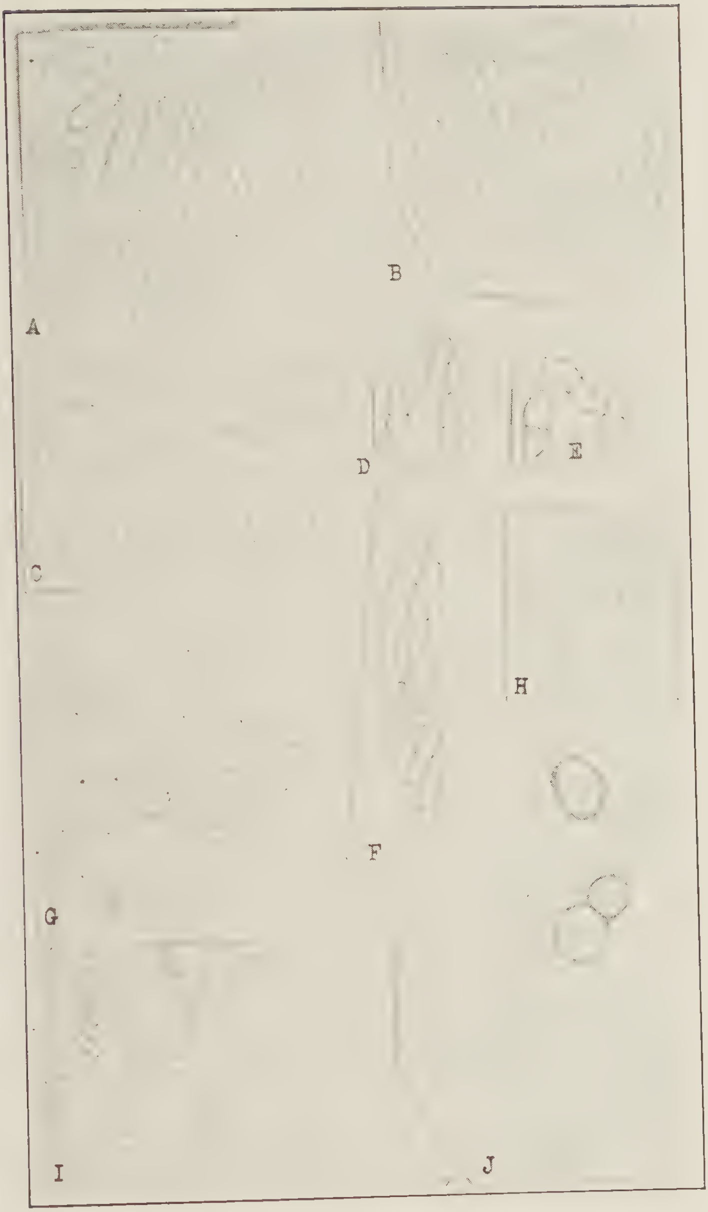

Figure 7. A. Pycnospores of Phoma medicaginis and B. pycnospores of phoma trifolii from potato dextrose agar cultures. C. Pycnospores of Aschochyta lethalis grown on sterile red clover leaves. A, B and C X1650. D. Pycnospores, E, ascospores and F, an ascus of Mycosphaerella lethalis, F. R. Jones' culture 921, grown on sterile red clover leaves. X750. G. Detail drawing of pycnospores of P. medicaginis from potato dextures agar culture. X 1650. H. and I. Pycnospores of P. medicaginis from overwintering alfalfa stems, showing occasional septate spores. J. Pycnospores and chlamydospores of P. trifolii from potato dextrose agar culture. X 750. The same type of chlamydospores is produced by P. medicaginis and Mycosphaerella lethalis.

while the spores of the alfalfa organism are distinctly cylindrical or oblong (Figure 7 A, B).

An ascigerous stage of the fungus has not been found. *Pleospora, Ophiobolus,* and *Leptosphaeria* were found fruiting on the overwintering stems of alfalfa but single-spore cultures of each resulted in cultures distinct from *Phoma.*

The identity of the organism is uncertain. It may be *Phoma herbarum* West. (Saccardo III, p. 133) but as this species includes Phomas from other genera of plants it can hardly be considered a good species. The same objection applies to *Phoma vulgaris* Sacc. (Saccardo III, p. 119). It is probably the same as *Phyllosticta medicaginis* (Fuckel) Sacc. (Saccardo III, p. 42), altho this is described as having curved spores while those of the alfalfa black-stem organism are rarely curved. *Phoma medicaginis* Malbr. et Roum. (Rev. Myc. 1886, p. 91) was described from alfalfa stems, the description being as follows: ''3675. *Phoma medicaginis* Malbr. et Roum. sp. nov. (Ph. Herb. f. medicaginis in Herb.) differe du type par des sporules oblongues, hyalines, plus petites,. 3–7=1–2½. Sur les tiges seches du *Medicago sativa,* Rouen (Seine-Infer.) 6 octobre 1885 (A. Malbranches).'' This description fits the black-stem organism closely enough to leave little doubt that they are the same. It is probable that *Phoma herbarum* f. *medicaginis* Fuckel, *Phoma medicaginis* Malbr. and Roum, and *Phyllosticta medicaginis* (Fuckel) Sacc. are synonymous. As the black-stem organism appears to be *the Phoma* and *Phyllosticta* of alfalfa which is found at all times of the year on both leaves and stems, it seems that it might properly be referred to either *Phoma medicaginis* Malbr. and Roum., or to *Phyllosticta medicaginis* (Fuckel) Sacc. As the organism is an inhabitant of the stems the year around, overwintering on dead stems and spreading from them to leaves and new stems the name *Phoma medicaginis* Malbr. and Roum. seems preferable to the writers.

Red Clover. Phoma was isolated from leafspots, petiole spots, petioles which were dying back after death of the leaflets in winter, blackened stems, crowns of dying plants, from decaying rootlets near the surface of the soil, and from pycni-

dia on dead overwintering stems. It was isolated at all seasons of the year and appears to be ever present on red clover in this State. It is readily isolated from affected parts. It forms a pure white to pale pink nearly resupinate irregularly concentric growth for several days, when the older portion begins to darken. Older cultures are covered irregularly with grayish-white aerial masses of hyphae, the substratum turning black. Chlamydospores are produced either singly or in short chains. Pycnidia are borne sparingly except in an occasional culture where they may be abundant. Pycnidia are borne on inoculated dead leaves or on naturally infected leaves placed in a damp chamber. They appear identical with those of the alfalfa organism. Spores are extruded in a gelatinous mass when the pycnidia are wet on a slide. In tubes they are extruded as light, grayish-pink droplets. The spores measure 4 x 1.8 to 7 x 3 microns, are oval, and of a more uniform size than those of the alfalfa organism. Crystals are produced in potato 2 percent dextrose agar, altho three cultures of more than one hundred separate isolations have never been observed to produce them.

Pleospora perithecia are found associated with *Phoma* pycnidia on dead stems but so far no proof of a genetic connection has been established.

Saccardo (X, p. 128) lists one species, *Phyllosticta trifolii* Rich., the spores of which are only 2-3 microns long, on *Trifolium repens* L., and no species of *Phoma* on *Trifolium.* Seymour (5) lists no Phomas or Phyllostictas on *Trifolium* in North America. The red clover organism differs from *Phoma medicaginis* in spore shape, cultural characteristics, and in the rate at which it darkens affected tissue. As isolations from the two hosts are consistently different the red clover organism must be considered as distinct from *Phoma medicaginis* and the name *Phoma trifolii* is suggested. A description follows:

Phoma trifolii n. sp. Spots irregular in size coalescing to form black streaks or to completely blacken portions of the stem and petiole; smooth, not depressed, except on younger tissue; in spring tender shoots of unadapted varieties often killed, leafspots abundant following warm, rainy periods in winter and

spring. Pycnidia few or absent on affected stem while alive; developing on dead shoots during winter and on recently killed dead shoots in the spring; usually spherical, scattered, immersed, then erumpent, dark brown to black, 150 to 320 microns on sterile clover leaves, smaller on dead stems, opening by a nearly circular ostiole. Spores hyaline, one or two guttulate, somewhat variable in size, oval, from 4 x 1.8 to 7 x 3 microns, usually continuous. In culture heavy-walled, smooth, multiguttulate, light to dark brown spherical or compressed chlamydospores 7 to 15 microns in diameter. In potato-dextrose-agar cultures dendritic groups of needle-shaped crystals are formed. Habitat. On leaves, petioles, stems and occasionally roots of *Trifolium pratense* L. in Kentucky and other parts of the United States.

Sweet clover. Ascochyta has been isolated from black lesions on sweet clover stems, from light yellow leafspots and from darkened petioles (Figure 5). It was found fruiting abundantly on overwintering stems of sweet clover. Perithecia of *Mycosphaerella* are frequently found intermingled with pycnidia of *Ascochyta* on dead stems in the spring. Single ascopore cultures from dead stems have produced an *Ascochyta* identical with that isolated from black lesions on stems, from leaves, and from overwintering stems. A culture of *Ascochyta* transferred to steam-sterilized red clover leaves in a culture dish developed both pycnidia of *Ascochyta* and perithecia of *Mycosphaerella* (Figure 7, D, E, F,). A comparison of this organism with *Mycosphaerella lethalis* Stone, indicates that the latter fungus is the cause of the black-stem disease of sweet clover. In common with *Phoma medicaginis and Phoma trifolii, Mycosphaerella lethalis* produces crystals on potato dextrose agar, but not so consistently. Potato dextrose agar cultures of *Mycospharella lethalis* appear identical with cultures of *Phoma trifolii* making a white, nearly resupinate, irregularly concentric growth on slants for several days before the older portions of the culture darken. Eventually the production of the larger septate spores in pycnidia distinguish them.

Stone (6) described the lesions of *Mycosphaerella lethalis*

as being elliptical with a grayish-white center and a brown, raised border. This type of lesion seems to occur when the outer tissues split open. If the cuticle and epidermis remain intact, blackening results, with failure of pycnidial production. Thus the two types of symptoms appear to be caused by the same organism.

Figure 8. Black-stem of sweet clover showing stem, petiole and leaf infections. This represents typical results obtained in infection experiments with each of the three organisms on the three crops. Plant inoculated March 6, 1933, with a culture of Phoma medicaginis. Photographed March 20, 1933.

INOCULATIONS

The results of inoculations with *Phoma medicaginis, P. trifolii*, and *Mycosphaerella lethalis* are given in table 4. Inocula-

tions were made by crushing an agar culture of the organism in water and rubbing this decoction on the stems and leaves of the plant to be inoculated, with a sterile swab made of a stick on the end of which was wrapped a piece of cheesecloth. The host was usually slightly wounded. Each of the three organisms was pathogenic on each of the three host species, producing the typical stem-blackening and leaf-injury (Figure 8.)

Phoma medicaginis regularly produced blackening of the stems of the three hosts more rapidly than the other two organisms with the exception of two cultures of *Phoma trifolii*. These, in common with *P. medicaginis*, produced pycnidia abundantly in culture suggesting that an abundance of spores, with this method of inoculation, is necessary for the rapid appearance of the blackening. Pycnidia were never observed on inoculated plants in the greenhouse.

Each organism was reisolated from black lesions on inoculated plants many times. The figures given in the last column of table 4 merely indicate the number of plants from which reisolations were made. Reisolated cultures were used as inoculum and found to produce the typical lesions on each of the host plants. While each organism appears to cause infection on the three distinct hosts, no evidence has been obtained in numerous isolations from natural infections that other than the specific parasite is concerned in blackening of the stems on a given host plant, altho what appears to be *P. trifolii* has been isolated from old dead stems of sweet clover and inoculations have produced typical black stem lesions on sweet clover. Evidently there is a high degree of adaptation between parasite and host which prevents these organisms from becoming extensively established on other host plants, even tho these are susceptible to artificial infection.

DISCUSSION

Legumes are essential in a cropping system if soil nitrogen is to be increased or even maintained. At one time red clover was the most commonly grown legume in this State. With the introduction of foreign red clover seed, failures became increasingly frequent but lack of adaptation was not commonly rec-

ognized as the cause. Because of the uncertainty of red clover other legumes were tried.

Sweet clover was found to be a valuable crop in several north-central Kentucky counties where liming was not necessary, and is being grown now in other sections of the State after liming. However, failures have now become frequent

TABLE 4. Record of inoculations of alfalfa, red clover and sweet clover with Phoma medicaginis, P. trifolii and Mycosphaerella lethalis.

	Inoculations to alfalfa.	Inoculations to red clover	Inoculations to sweet clover	Reisolations from
Eight cultures of P. medicaginis from living alfalfa stems and petioles, isolated from Apr. to June.	17*/17	11/11	3/3	4 plants
Ten single-spore cultures of P. medicaginis from pycnidia on dead stems of alfalfa, isolated in March, April and May.	6/6	12/12	6/6	5 plants
Five cultures of P. medicaginis isolated by F. R. Jones from alfalfa from Wisconsin, Nebraska, and Mississippi.	6/6	3/2	10/10	1 plant
Thirteen cultures of P. trifolii isolated from red clover leaves, stems, petioles and crowns.	4/4	23/23	18/13	1 plant
Nine cultures of M. lethalis from sweet clover from Kentucky, Wisconsin, Illinois and Ohio.	16/8	10/5	21/13	2 plants

* Numerators represent the number of plants inoculated and the denominators the number of plants which developed black-stem.

enough to be discouraging to all but a few farmers. Nevertheless its value in preparing hilly land for grass has been fully demonstrated. Alfalfa was next tried. It was used as a profitable cash crop for a time, but sudden losses of stand and dif-

ficulty in re-establishing stands where it had previously been grown, especially on eroded hill lands made its use rather an uncertain undertaking. More recently the various annual lespedezas have been introduced and are now being widely grown, but they should hardly be expected to fill the place of such valuable crops as red clover, sweet clover, and alfalfa if these can be grown successfully.

Red Clover. Black-stem disease appears to destroy some varieties of red clover in Kentucky, especially those from Europe. In certain years other diseases, as southern anthracnose, and stem or crown rot, appear to be the primary cause of failure. Southern anthracnose has probably been credited with causing more failures of unadapted clovers in the southern part of the United States than it deserves. With an increasing knowledge of the causes of clover failure in Kentucky, the large part which *Phoma trifolii* plays becomes increasingly obvious. While it works slowly, it appears to be everpresent and persistent in its activity so that its cumulative effect is great. The obvious means of control of a disease of this type is by the selection of resistant varieties. Fortunately, thanks to the intelligence and persistence of a few farmers, red clover varieties sufficiently resistant to cold and the more destructive diseases, have been developt in Kentucky. These have proved to be resistant to *Phoma trifolii* and persist where unadapted clovers are killed. Recognition of the fact that *Phoma trifolii* may be one of the causes of failure of unadapted clover helps to clarify the problem as to what constitutes an adapted strain of red clover for Kentucky.

Alfalfa. Alfalfa failure in Kentucky appears to be a complex problem but perhaps not so complex as red clover failure. *Sclerotinia trifoliorum* may reduce the stand of fall-sown alfalfa the first spring, but subsequently seems to cause little injury. The part which cold plays in failure has not yet been properly evaluated but the Grimm and variegated types appear to be less injured by spring freezes than some common sorts. Leaf hoppers are frequently very injurious to the crop, especially in early summer. Black-stem appears to be a major cause of

gradual reduction in stands, and in some seasons appears to destroy stands almost completely.

Resistance to black-stem injury seems inherent in some varieties of alfalfa. Differences in two varieties in degree of injury by *Phoma medicaginis* and in stand remaining after several years were noticed in a few fields. While no records of the exact sources of seed are available, yet the fact seems established that all varieties are not equally injured by black-stem. As alfalfa seed does not set readily in Kentucky, the solution of this phase of the problem depends upon trials of numerous varieties of alfalfa, the sources of which are known, in order that seed of resistant strains may be found.

Failure of a well established stand of alfalfa not only involves the loss of the crop but also soil depletion by sheet washing, lowered fertility caused by continued cropping and, frequently, failure to reestablish a stand on the same land. Loss from alfalfa failure could be greatly reduced, especially in the hill region of north-central Kentucky, if bluegrass were sowed with alfalfa, for with gradual or sudden failure of the alfalfa crop, the land would be in grass. It has been observed that where bluegrass has gradually come into alfalfa fields, the alfalfa died out first and was not crowded out as farmers commonly believe.

Grazing alfalfa seems to provide a promising means of reducing loss of stands from black-stem. The object of grazing is to break down the brittle, dead shoots bearing inoculum so that the tender shoots which develop in the spring will not be subjected to such heavy infection. A single experiment was made in which sheep grazed alfalfa, beginning shortly before growth commenced in the spring and continuing until the alfalfa in the ungrazed portion of the plot was about 5 inches high. The sheep, in eating the tender alfalfa shoots, broke off practically all dead material. The grazed alfalfa was soon as tall as the ungrazed. The stems were more uniform in height in the grazed portion and it was freer from weeds. The stems were practically free from discoloration and leaf injury was slight. In the ungrazed portion there was a heavy undergrowth of

winter weeds, shoots continued to die thruout the spring, and leaves were killed about half way up the shoots. Considerable blackening of the shoots occurred. The shoot injury recorded on page 62 (Figure 1), occurred in the ungrazed portion of this field. If loss of alfalfa stands is due, as seems probable, to inoculum on standing dead shoots, then grazing should provide a means of reducing injury from black-stem and of improving the quality of the first cutting of alfalfa.

Sweet Clover. The part which the black-stem disease plays in sweet clover failures in Kentucky is not fully known. It is probable that it causes extensive injury only in exceptionally wet seasons and then probably does not account for complete failures of second-year clover which sometimes occur in wet springs in north-central Kentucky. Observations of the extent of injury to individual plants of sweet clover leave little doubt that differences in resistance to *Mycosphaerella lethalis* occur and that thru selection much can be done toward the development of sweet clover varieties adapted to Kentucky conditions. Even with a complete failure of a sweet clover stand the second spring, the loss is not so great if grass has been sown and a stand obtained. Nearly the maximum nitrogen content is present in sweet clover plants soon after growth commences the second spring and if the plants are killed, this nitrogen becomes available to the grass crop in a short time.

SUMMARY

1. Black-stem of alfalfa, red clover and sweet clover are diseases of economic importance in Kentucky and probably in other humid areas where the winters are mild and the spring season is long and likely to be wet. The disease has previously been given little attention as a cause of failure of these crops. Spring-sown stands of unadapted varieties of red clover may be destroyed completely when about a year old, the injury apparently being cumulative from the previous year. In favorable seasons for the development of the disease, alfalfa stands are greatly reduced and sometimes destroyed. The injury to sweet clover is sometimes extensive. It is not known whether

this disease is the cause of complete failure of sweet clover stands which frequently occurs in Kentucky.

2. Black-stem of alfalfa is caused by a species of *Phoma* which appears to be identical with *Phoma medicaginis* Malbr. and Roum. and probably with *Phyllosticta medicaginis* (Fuckel) Sacc. The former name is preferred by the authors. Black-stem of red clover is caused by a species of *Phoma* which does not appear to have been described. The name *Phoma trifolii* n. sp. is suggested and a description is given. Black-stem of sweet clover is caused by what appears to be *Mycosphaerella lethalis* Stone.

3. Greatest injury is caused to each of the three crop plants soon after growth commences in the spring. Leaves are killed by leafspot and the tender shoots die either from direct infection or because the leaves are killed. Blackening of the stems, which is evident late in the spring, is the most readily observed symptom but causes comparatively little injury.

4. Red clover which has been grown continuously in Kentucky for many years appears to be little injured by *Phoma trifolii.* The same is true of varieties from other Southern states. Northwestern clovers are sometimes considerably injured and European varieties seem to be very susceptible. Alfalfa varieties seem to show distinct differences in degree of injury by *Phoma medicaginis.* Individual sweet clover plants show marked differences in degree of injury by *Mycosphaerella lethalis.*

5. The Phomas on red clover and alfalfa are active during warm periods in winter, causing leaf and petiole spots. The organisms also overwinter in dead stems of the respective crops. Pycnidia are produced in February and March. *Mycosphaerella lethalis* overwinters in dead sweet clover stems where pycnidia and perithecia are produced in early spring. Pycnidia of each of the organisms are produced on spotted leaves if put in a damp chamber and are produced on recently killed tender shoots.

6. Blackening of stems of alfalfa and red clover is not confined to the spring crop but will develop during the summer

if the crops are allowed to stand beyond the time of cutting for hay.

7. Loss from black-stem of red clover is prevented thru planting adapted varieties of red clover. Injury to alfalfa stands appears to be greatest where the last crop of alfalfa is allowed to stand in the fall, thus providing abundant inoculum the following spring. Destruction of this material by winter grazing or by other means offers promise of reducing losses of stand during the spring.

BIBLIOGRAPHY

1. Corneli, E. Sopra una grave alterazione della Medica prodotta da 'Phyllosticta medicaginis' Fuck. a Perugia. Rev. Pat. Veg. 22: 3-4, pp. 51-58, 1932. See R. A. M. 11: pp. 653-654, 1932.
2. Fergus, E. N. An analysis of clover failure in Kentucky. Ky. Expt. Sta. Rsch. Bul. 324, 1931.
3. Horsfall, James G. A study of meadow-crop diseases in New York. Cornell Agr. Expt. Sta. Memoir 130, 1930.
4. Rosella E. Observations sur l'Ascochyta de la Luzerne. Rev. Path. Veg. et Ent. Agric. 16: 8, pp. 226-229, 1929. See R. A. M. 9: 249, 1930.
5. Seymour, Arthur Bliss. Host index of the fungi of North America. Harvard University Press, 1929.
6. Stone, R. E. The life history of Ascochyta on some leguminous plants. Annales Myco. 10: pp. 564-592, 1912.
7. Valleau, W. D. and E. N. Fergus. Black-stem disease of alfalfa, sweet clover and red clover. Phytopathology 19: 507-508, 1929.